A comparative analysis of ISSR and RAPD markers for studying genetic diversity in Iranian pistachio cultivars

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Abstract
Pistachio is one of the most important horticultural products of Iran. The best way to get the maximum yield is to have genetically pure and monotonous gardens. Thereby, study of genetic variation and providing genetic identifications, make this possible to have homogenous gardens with high performance genotypes. In the present study, genetic diversity of 19 Iranian pistachio cultivars was assessed using ISSR and RAPD primers. Among these markers, RAPDs generated 127 amplification products, out of which 88 were polymorphic and ISSRs produced 114 amplification products, out of which 73 were polymorphic. RAPD fingerprinting detected more polymorphic loci (67%) than the ISSR (63%). Mean of polymorphism information content (PIC) for each of the marker systems (0.39 for RAPD and 0.39 for ISSR) suggested that both the marker systems were equally effective in determining polymorphisms. The dendrograms constructed using RAPD and ISSR marker systems were highly correlated with each other as revealed by high Mantel correlation (r= 0.83). These two marker systems were found to be useful for genetic diversity studies in pistachio and identify variation within *Pistacia vera* cultivars.

Key words: Pistachia, fingerprinting, horticultural product, molecular maker.

Introduction

Pistachio (*Pistacia vera* L.) is one of the most important horticultural products of Iran. It is a diploid (2n = 30) member of the Anacardiaceae family. The genus Pistacia consists of eleven species that *P. vera* is the only cultivated and commercially grown species in this genus (Zohary 1996). The other species grow as wild and their seeds are used mainly as a rootstock seed source. Due to its drought and salinity resistance and ability to growth in weak soils, this plant can be cultivated in desert-like and dry lands. It is cultivated in many places around the world, Asia, Middle East, southern Europe, South Africa, Australia and America. The main world producers of pistachio nuts are Iran, USA, Turkey and Syria (FAO, 2006).
The best way to get the maximum yields is related to have genetically pure and monotonous gardens. Thereby, the study of genetic diversity and providing genetic identification, make this possible to have homogenous gardens with high performing genotypes. Initial classification of Pistacia species was carried out morphologically based on tree, leaf, flower and nut characteristics. Isozyme markers have also been used to investigate the genetic diversity of pistachios (Rovira *et al.*, 1995). DNA markers have proved to be an efficient tool for the molecular characterization of the plant species. The classification of Pistacia species at molecular level was first done based on chloroplast DNA profiles by Parfitt and Badenes (1997). RAPD has been the

most used method in pistachio to study genetic diversity and relationship among Pistacia species and cultivars (Hormaza *et al.*, 1994, 1998; Kafkas *et al.*, 2002; Katsiotis *et al.*,, 2003; Golan-Goldhirsh *et al.*, 2004; Mirzaei *et al.*, 2005; al-saghir *et al.*, 2006). Also AFLP, SSR, ISSR and comparisons of these markers were the other used markers in pistachio cultivars characterization (Katsiotis *et al.*, 2003; Ahmad *et al.*, 2003; Golan- Goldhirsh *et al.*, 2004; Ahmad *et al.*, 2005; Kafkas *et al.*, 2006; Ahmadi-Afzadi *et al.*, 2007; Ibrahim-Basha *et al.*, 2007; Salehi Shanjani *et al.*, 2009; fares *et al.*, 2009).

RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequences, which are able to differentiate between genetically distinct individuals. Inter simple sequence repeat (ISSR) is a RAPD like marker system. It can reveal variation in the numerous microsatellite regions by using primers that may be anchored with one or two nucleotides on either the 5' or 3' end of a repeat region and extend into the flanking region. These methods are widely applicable because they are rapid, inexpensive, and simple to perform, do not require prior knowledge of DNA sequence, and require very little starting DNA template (Esselman *et al.*, 1999).

In this study two markers, the random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) and inter simple sequence repeat (ISSR) (Vos *et al.*, 1995) were used to explore the genetic diversity among 19 Iranian genotypes of *pistachia vera* and were compared for their relative efficiency.

Materials and Methods

Plant Material and DNA Extraction

In the present study, genetic relationships and diversity among nineteen cultivars were assessed using RAPD and ISSR primers. Plant material for this study was collected from Iranian Pistachio Resources Institute (IPRI) (Table 1).

Total genomic DNA was extracted from freeze-dried leaf tissue using (CTAB) mini-extraction protocol based on the method by Hormaza *et al.*, (1998), with minor modifications. Quality and quantity of DNA were estimated by spectrophotometer and electrophoresis on 0.8% agarose gel. DNA samples were diluted to 25 ng/µl for polymerase chain reaction (PCR) amplification.

RAPD Amplification

Twenty RAPD primers obtained from Metabion Company were tested and 10 were used (Table 2). Amplifiction reactions were performed in 25 µl volume containing 13.5 µl PCR master kit, 8.5 µl dH_2O, 1 µl template DNA and 2 µl primer. PCR reactions were performed with thermal cycler (model TC-512). Amplification condition was an initial denaturing at 94°C for 5 min followed by 45 cycles of 1 min denaturing at 94 °C, 1 min annealing at 31°C -34 °C, and 2 min extension at 72 °C plus 7 min final extension at 72°C. PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gel in 1x TBE buffer, gels were stained with ethidium bromide and digitally photographed under ultraviolet light in a transilluminator documentation system (UVP, USA).

ISSR Amplification

PCR amplification was performed with primers obtained from Metabion Company. The experiment was carried out by 20 ISSR primers, of which 10 were used (Table 2). Amplification reactions were done in a 25 µl volume containing 12.5 µl PCR master kit, 10 µl dH_2O, 1µL template DNA and 1.5 µl primer. The mixture was overlaid with mineral oil and subjected to PCR on a thermal cycler (model TC-512), programmed for an initial denaturing of 5 min at 94°C, followed by 35 cycles of 30 sec denaturing at 94°C, 1 min annealing at 51°C -59°C, 2 min extension at 72°C, plus a 5 min final extension at 72°C. PCR products were analyzed by gel electrophoresis in 1.5% agarose in 1x TBE buffer, gels were stained with ethidium bromide and digitally

Table 1. Names and codes of nineteen Iranian pistachio cultivars, used in this study .

Cultivar code	Cultivar name	Cultivar code	Cultivar name
P1	Akbari	P11	Gholamrezai
P2	Ahmad-aghai	P12	Khanjari-damghan
P3	Kale-ghochi	P13	Sefid-peste
P4	Ohadi	P14	Lavare 2
P5	Ghazvini	P15	Fandoghi-riz
P6	Mohseni	P16	Ebrahim-abadi
P7	Javad-aghai	P17	Seifedin
P8	Amiri	P18	Rezai-zodras
P9	Sheni	P19	Momtaz
P10	Hosein-khani		

Table 2. ISSR and RAPD primer sequences and annealing temperatures (Ta).

ISSR			RAPD		
Primer	**Sequence (5'-3')**	**Ta °C**	**Primer**	**Sequence (5'-3')**	**Ta °C**
K 10	$(AC)_8YG$	54	Z 16	TCCCCATCAC	31
K11	$(CA)_6AG$	59	29	CCGGCCTTAC	34
K13	$(AG)_8YT$	54	28	CCGGCCTTAA	34
K15	$(GT)_8YC$	57	1	CCTGGGCTTC	34
K 16	$(CA)_8RC$	53	51	CTACCCGTGC	34
K25	$(AG)_8G$	54	OPAC-06	CCAGAACGGA	32
K26	$(AG)_8T$	53	OPA_11	CAATCGCCGT	32
K24A	$(GA)_8A$	53	OPA-04	CACAGAGGGA	32
K24B	$(CA)_8T$	51	Z 06	GTCCCGTTCA	33
UBC 840	$(GA)_8TT$	53	P 6	TCGGCGGTT	34

photographed under ultraviolet light in a transilluminator documentation system (UVP, USA).

Data Analysis

The amplifications were independently repeated three times using the same procedure, in order to ensure that the amplifications obtained with the primers were reproducible and consistent. The amplified bands were scored manually as 1 (present) and 0 (absent). A binary matrix was obtained by visual scoring of the bands in the cases of both RAPD and ISSR. Data analyses were performed using NTSYS-pc version 2.0 program package. Mantel (1967) test was used to measure the degree of relationship between similarity index matrices produced by any two-marker systems. Clustering dendrograms were constructed by un-weighted pair group method using arithmetic average (UPGMA) method. The polymorphism information content (PIC) of each marker that provides an estimate of the discriminatory power of a locus or loci was calculated by taking into account not only the number of alleles that are expressed but also relative frequencies of those alleles (Kumar *et al.*, 2003). Also marker index (MI), which is the product of polymorphic information content (PIC) and effective multiplex ratio (EMR), was used to evaluate the overall utility of each marker system. EMR (E) is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay.

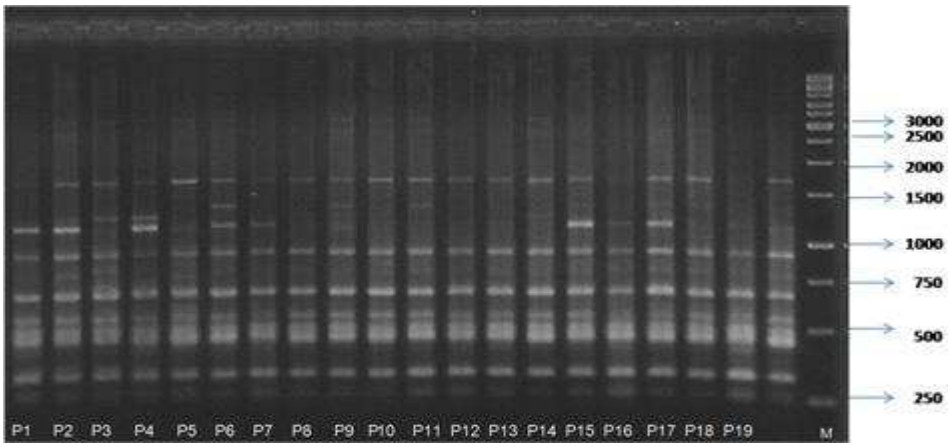

Fig 1. Genomic DNA amplification pattern in 19 pistachio cultivars, with ISSR primer (primer K26). (**M** molecular weight marker).

Table 3. The total number of loci, polymorphic loci, polymorphism percentage, PIC and MI, revealed by ISSR and RAPD primers.

Primer code		NO. locus	polymorph Locus	Polymorphism percentage	PIC	Emr	MI=PIC*E
ISSR	k10	12	11	0.92	0.39	6.71	2.64
	k16	8	3	0.37	0.39	2.37	0.94
	k11	12	9	0.75	0.45	5.92	2.66
	k15	9	7	0.78	0.46	4.34	1.98
	k13	11	7	0.64	0.39	5.56	2.19
	k26	12	7	0.58	0.40	5.10	2.02
	k25	15	8	0.53	0.40	6.71	2.70
	k24a	12	7	0.58	0.27	5.56	1.51
	k24b	11	6	0.55	0.34	4.59	1.56
	UBC840	12	8	0.67	0.43	6.38	2.73
RAPD	Z 16	7	5	0.71	0.32	3.87	1.26
	OPAC-06	11	9	0.82	043	6.20	2.68
	28	22	19	0.86	0.40	13.14	5.27
	1	16	10	0.63	0.30	7	2.12
	OPA-11	9	6	0.67	0.39	4.67	1.80
	OPA-04	8	4	0.5	0.28	3.63	1
	Z 06	11	8	0.73	0.41	4.98	2.05
	P 6	14	10	0.71	0.45	5.83	2.61
	29	14	7	0.5	0.39	5.24	2.07
	51	15	10	0.67	0.36	6.56	2.37

PIC: Polymorphic information content.
MI: Marker index.

Results

Out of 20 ISSR and 20 RAPD primers, only 10 ISSR and 13 RAPD primers produced DNA fragments which were scorable. All the 10 scorable ISSR primers were polymorphic and reproducible, while out of 13 RAPD primers, only 10 were polymorphic and reproducible.

ISSR banding pattern

The 10 chosen ISSR primers produced various numbers of DNA fragments, depending on their simple sequence repeat motifs. A total of 114 fragments were produced, out of which, 73 (64.03%) were polymorphic (Fig. 1). The size of the amplified products ranged from 250 bp to 2 kb. The range of polymorphism was between (37%) for K16 primer up to (91%) for K10 primer. The polymorphic information content (PIC) varied from 0.27 (K24a) to 0.45 (K15) with an average PIC of 0.39. The marker index (MI) varied from 0.93 (k16) to 2.72 (UBC 840) with an average of 2.09. Therefore the most informative primers were K11, K25 and UBC 840.

The number of polymorphic loci varied from 3 (K16) to 11 (K10) with an average of 7.3 per primer (Table 3).

RAPD banding pattern

In total, 127 loci were produced out of which, 88 loci (69%) were polymorphic (Fig. 2). All the selected primers amplified DNA fragments in the 19 genotypes studied, with the number of polymorphic locus varying from 4 (OPA-04) to 19 (28) with an average of 8.8 per primer (Table 3). The size of amplified products ranged from 250 bp to 2.5 kb. The range of polymorphism was between 50% for primers 29 and OPA-04 up to 86% for primer 28. The polymorphic information content (PIC) varied from 0.28 (OPA-04) to 0.45 (P6) with an average PIC of 0.39. The marker index (MI) of primers varied from 1(OPA-04) to 5.26 (28) with an average of 2.32. The most informative primers were 28 and OPAC-06.

Grouping the cultivars

RAPD

Estimates of genetic relationships were obtained from the markers data using Jaccard's similarity coefficient. According to the results, genetic similarity ranged from 0.56 (low similarity) between Amiri and Javad-aghai up to 0.79 (high similarity) between Gholamrezai, Khanjari-damghan and Ebrahim-abadi. Cluster analysis revealed 4 main groups (Fig. 3a). Cluster I consisted of Gholamrezai, Ahmad-aghai, Kale-gochi, Ohadi, Hosein-khani, Rezaei-zodras, Seifoddin. Cluster II consisted of Mohseni, Fandoghi-riz and Cluster III was comprised of Ghazvini, Momtaz. The last group included Sheni and Ebrahim-abadi. The result of principal component analysis (PCA) was comparable to the cluster analysis and the first three most informative PC components explained 76.1 % of the total variation (Fig. 3a).

ISSR

The genetic similarity matrices were constructed using Jaccard's similarity coefficient. According to the results, genetic similarity ranged from 0.53 (low similarity) between Kale-ghochi, Hosein-khani and Ebrahim-abadi up to 0.83 (high similarity) between Kale-ghochi and Ahmad-aghai. Cluster analysis using UPGMA revealed three main groups with 13, 2 and 4 pistachio varieties in each group (Fig. 3b). Cluster I consisted of Akbari, Gholamrezai, Ahmad-aghai, Kale-gochi, Ohadi, Hosein-khani, Fandoghi- riz, Sheni, Lavare 2, Rezaei-zodras, Seifoddin and Momtaz (Fig. 3b). Cluster II consisted of Ghazvini and Sefid-peste and Cluster III was comprised of Mohseni, Ebrahim-abadi, Amiri, Khanjari-damghan (Fig. 3b). The result of principal component analysis (PCA) was comparable to the cluster analysis. The first three most informative PC components explained 78.4 % of the total variation.

Combined (ISSR and RAPD) data

Combining both markers, a total of 241 DNA fragments were produced, of which 161 of them (85.8%) were polymorphic with an average of 5.45 polymorphic fragments per primer. By combining markers data (RAPD + ISSR), the similarity coefficient varied from a minimum of 0.56 (Hosein-khani and Ebrahim-abadi) to the maximum of 0.81 (Ohadi and Kale-gochi) with an average similarity value of 0.68. The Mantel test between the two Jaccard's similarity matrices gave r = 0.83 that indicated very

good agreement between RAPD and ISSR marker systems.

The genetic relatedness of cultivars studied through RAPD and ISSR markers, showed similar dendrogram topologies with some exceptions. However, the general dendrogram constructed using Jaccard's similarity coefficient values of combined molecular data (RAPD + ISSR) revealed a better representation of the relationship than individual markers. The cultivars grouped into 3 main clusters and some of the clusters divided further into sub-clusters (Fig. 3c). A single cultivar, Ebrahim-abadi formed a distinct cluster and showed a distant relationship with Akbari (Fig. 3c).

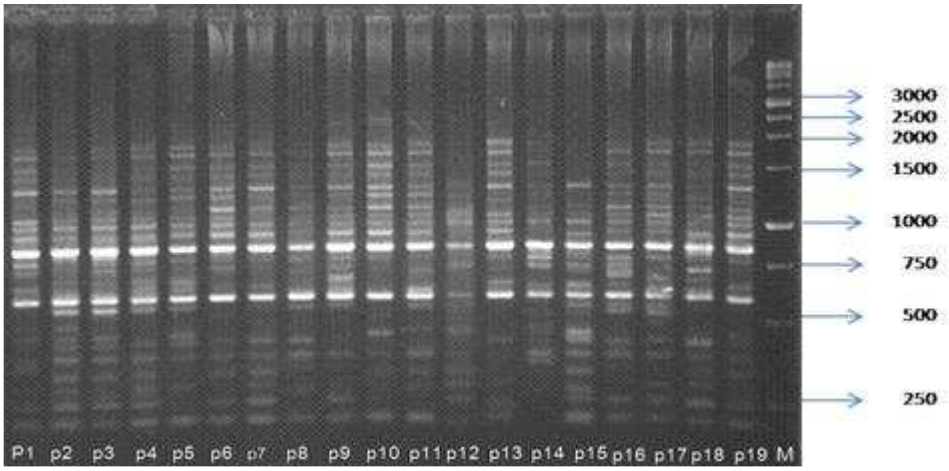

Fig 2. Genomic DNA amplification pattern in 19 pistachio cultivars, using 10-mer RAPD primer (primer 28) (M, molecular weight marker).

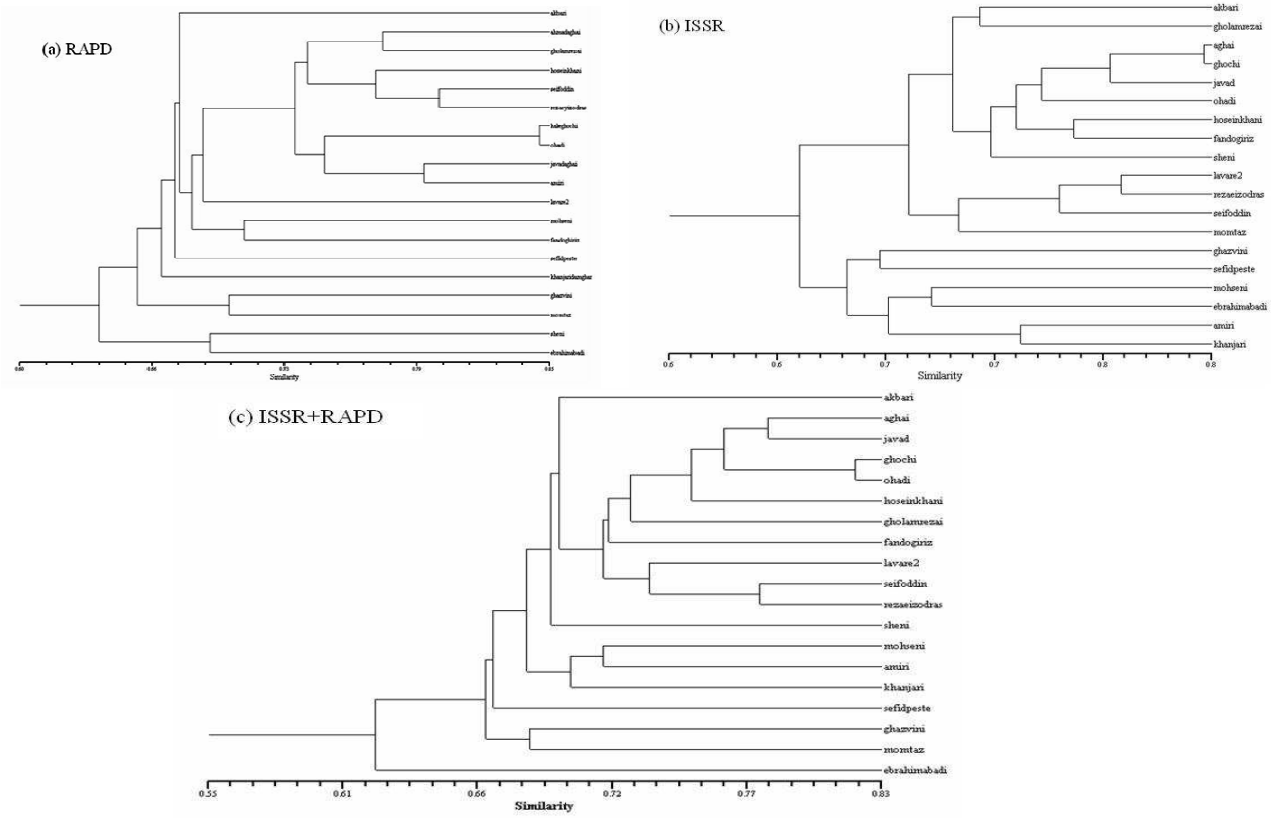

Fig 3. UPGMA dendrogram of the 19 pistachio cultivars, based on Jaccard's similarity coefficient Using (a) RAPD (b) ISSR and (c) ISSR + RAPD data.

Results indicated the presence of a wide genetic variability among different genotypes of Pistachio. The three-dimensional ordination confirmed the cluster analysis results and the first three most informative PC components explained 76.4% of the total variation (Fig. 4a-c). The

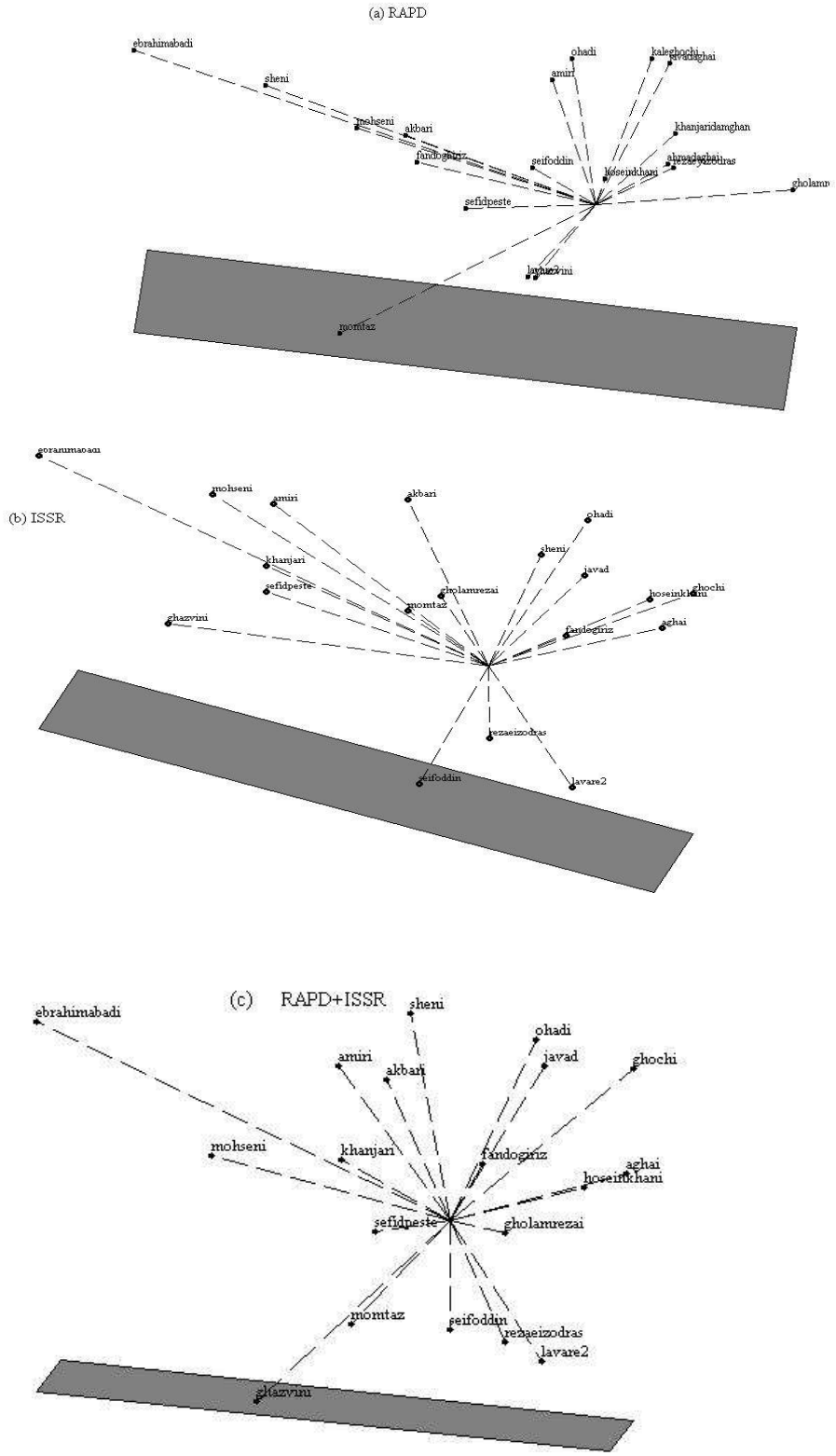

Fig 4. Three-dimensional plot of principle component analysis of 19 pistachio cultivars using (a) RAPD (b) ISSR (c) RAPD+ISSR data.

grouping of cultivars using three first PC components was in agreement with the results in clustering. It showed that Ebrahim-abadi is the most distant cultivar, Ghazvini and Momtaz clustered in the same group, Mohseni, Amiri and Khanjari-damgan in other group, lavare 2, Seifedin, Rezai-zodras, Gholamrezai, Hosein-khani, Fandoghi-riz, Ohadi, Javad-aghai and Kale-ghochi as the close cultivarss in the last group.

Discussion

Comparative studies in Pistacia species involving RAPD, AFLP and ISSR marker systems have been successfully used by very limited researchers (Kafkas *et al.*, 2006). In this work we compared the applicability of ISSRs and RAPDs as genetic markers for the study of genetic diversity in Iranian pistachio cultivars. Between the two marker systems employed, 10 RAPD primers produced a total of 127 DNA fragments, whereas 10 ISSR primers produced only 114 DNA fragments. The level of polymorphism revealed by RAPD (67%) was higher than ISSR (63%). In agreement with our results, Archak *et al.*, (2003) detected 87.9 % of polymorphism in 19 cashew accession with RAPDs and 73.7 % with ISSRs. Mohd-arif *et al.*, (1997) also reported similar results for Shisham (*Dalbergia sissoo*). It has been reported that the ability to resolve genetic variation may be more directly related to the degree of polymorphism detected by the marker system (Sivaprakash *et al.*, 2004). Comparison of PIC values for the two marker systems indicated that the range of PIC values for RAPD primers was from 0.27 (OPA-04) to 0.45 (P6). This is because of poly-allelic nature of RAPD markers. Also the comparison of the average PIC values of ISSR primers revealed that the lowest value was 0.27 with K24a and the highest value was 0.46 with k15. RAPD and ISSR markers grouped the 19 cultivars into four and three clusters. The clustering of genotypes within groups was similar with some exceptions. A possible explanation for the difference in the resolution of RAPD and ISSR is that the two marker techniques targeted different regions of the genome. These differences may also be attributed to marker sampling errors and/or the level of polymorphism detected, reinforcing the importance of the number of loci and their coverage of the whole genome for obtaining reliable estimates of genetic relationships among cultivars (Souframanien and Gopalakrishna 2004). The correlation between Jaccard's similarity values obtained from two marker techniques was high ($r > 0.83$). This indicates very good correlation between ISSR and RAPD based similarities. ISSR method has been reported to be more reproducible (Goulao and Oliveira 2001) which produces more complex marker patterns than the RAPD approach, and is advantageous when differentiating closely related cultivars. ISSR has also been used for cultivar identification. Nevertheless, on the basis of PIC values (RAPD=0.39; ISSR=0.39), percent of polymorphism (RAPD=67%; ISSR=63%), and similarity matrix, the RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in Iranian pistachio cultivars. Similar results are reported in Caldesia grandis (Chen *et al.*, 2006). This may be because of the fact that two marker techniques targeted different regions of the genome. Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Bachmann 1997; Landergott *et al.*, 2001). On the contrary, some other studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci (Penner 1996). However, there is little information to indicate that ISSR markers are functionally important (Esselman *et al.*, 1999).

The high similarity between Kale-gochi and Ohadi was noticed in both RAPD and combined analysis indicating that these genotypes are closely related. But high similarity of Kale-gochi and Ahmad-aghai was observed in ISSR marker system. Grouping by ISSR, showed the akbari and khanjari-damghan as most divergent ones while the Akbari and Ebrahim- abadi were most diverse using RAPD results. This revealed the existence of sufficient amount of genetic variability among the pistachio, which could be exploited further. A close genetic similarity was found in some of the cultivars analyzed as shown by high values of similarity index. Also, the similarities detected with ISSRs were greater than the similarities observed with RAPDs. Fernandez *et al.*, (2002) studied 16 barley cultivars form different countries and found a higher similarity index by ISSRs than by RAPDs. It may be due to highly polymorphic, abundant nature of the microsattelites due to slippage in DNA replication. In summary, the markers employed either individually or in combination were effective in discriminating the different types and may be useful for better management of germplasm resources.

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Rererences

Ahmad R., Ferguson L., Southwick S. M. (2003). Identification of pistachio (Pistacia vera L.) nuts with microsatellite markers. *Journal of the American Society for Horticultural Science*, 128: 898-903.

Ahmad R., Ferguson L., Southwick S. M. (2005). Molecular marker analyses of pistachio rootstocks by Simple Sequence Repeats and Sequence-Related Amplified Polymorphism. *Journal of Horticultural Science and Biotechnology*, 80: 382-386.

Ahmadi-Afzadi M., Sayed Tabatabaei B. E., Mohammadi S. A., Tajabadipur A. (2007). Comparison of genetic diversity in species and cultivars of pistachio (*Pistacia* sp. L.) based on Amplified Fragment Length Polymorphism (AFLP) markers. *Iranian Journal of Biotechnology*, 5: 147-152.

Archak S., Gaikwad A.B., Gautam D., Rao E.V.V.B., Swamy K.R.M., Karihaloo J.L. (2003). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L) accessions of India. *Genome*, 46: 362–369.

Bachmann K. (1997). Nuclear DNA markers in plant biosystematics research. *Opera Botany*, 132: 137–148.

Chen J.M., Gituru W.R., Wang Y.H., Wang Q.F. (2006). The extent of genetic diversity in the rare *Caldesia grandis* (Alismataceae): comparative results for RAPD and ISSR markers. *Aquatic Botany*, 84:301–307.

Dollo L., Hormaza J. I., Polito V. S. (1995). RAPD polymorphisms among pistachio (*Pistacia vera* L.) cultivars. *Fruit Varieties Journal*, 49: 147-152.

Esselman E.J., Li J.Q., Crawford D., Winduss J.L. Wolfe A.D. (1999). Clonal diversity in the rare Calamagrostis porteri ssp. In sperata (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. *Molecular Ecology*, 8: 443–45.

FAO. (2006). FAOSTAT database. http://apps.fao.org/page/form.

Fares K., Guasmi F., Touil L., Triki T., Ferchini A. (2009). Genetic diversity of Pistachio tree using inter-simple sequence repeat markers(ISSR) supported by morphological and chemical markers. *Biotechnology*, 8: 24-34.

Fernandez M.E., Figueiras A.M., Benito C. (2002). The use of ISSR and RAPD markers for detecting DNA

polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theoretical and Applied Genetics*, 104: 845-851.

Goulao L., Cabrita L., Oliveira C.M., Leitao J.M. (2001). Comparing RAPD and AFLP analysis in discrimination and estimation of genetic similarities among apple (Malus domestica Borkh) cultivars. *Euphytica*, 119: 259–270.

Golan-Goldhirsh A., Barazani O., Wang Z.S., Khadka D.K., Saunders J.A., Kostiukovsky V., Rowland L.J. (2004). Genetic relationships among Mediterranean *Pistacia* species evaluated by RAPD and AFLP markers. *Plant Systematics and Evolution***,** 246: 9-18.

Gupta M., Chyi Y.S.I., Romero-Severson J., Owen J. L. (1994). Amplification of DNA markers from evolutionary diverse genomes using single primers of simple sequence repeats. *Theoretical and Applied Genetics*, 89:998–1006.

Hormaza J.I., Dollo L., Polito V.S. (1994). Determination of relatedness and geographical movement of *Pistacia vera* germplasm by RAPD analysis. *Economical Botany*, 48: 349-358.

Hormaza J.I., Pinney K., Polito V.S. (1998). Genetic diversity of pistachio (*Pistacia vera*) germplasm based on Randomly Amplified Polymorphic DNA (RAPD) markers. *Economical Botany*, 52: 78-87.

Kafkas S., Perl-Treves R., (2001). Morphological and molecular phylogeny of *Pistacia* species in turkey. *Theoretical and Applied Genetics*, 102: 908-915.

Kafkas S., Cetiner S., Perl-Trevis R. (2001). RAPD markers linked to sex in the genus Pistacia. *Journal of Horticultural Science and Biotechnology*, 76: 251-255.

Kafkas S., Ozkan H., Acar I., Atli H.S., Koyuncu S.A.k., Acar B.E., Atli I., Penner G.A. (1996). *RAPD analysis of plant genomes*. In: Jauhar PP(ed) Methods of genome analysis in plants. CRC, Boca Raton, pp 251–26.

Koyuncu H.S. (2006). Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: comparison of AFLP, ISSR, and RAPD markers. *Journal of the American Society for Horticultural Science*, 131: 522-529.

Katsiotis A., Hagidimitriou M., Drossou A., Pontikis C., Loukas M. (2003). Genetic relationships among species and cultivars of Pistacia using RAPDs and AFLPs. *Euphytica*, 132: 279-286.

Kumar P., Singh K., Vikal Y., Randhawa L.S., Chahal G.S. (2003). Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characteristics. *Indian Journal of Genetics*, 63: 5–10.

Landergott U., Holderegger R., Kozlowski G., Schneller J.J. (2001). Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. *Heredity*, 87:344–355.

Mantel, N. (1967). The detection of disease clustering and generalized regression approach. *Cancer Research*, 27: 209–220.

Mirzaei S., Bahar M., Sharifnabi B. (2006). A phylogenetic study of Iranian wild pistachio species and some cultivars using RAPD markers. *Acta Horticultura*, 726: 39-43.

Mohd A., Zaidi N.W., Singh Y.P., Rizwanul H., Singh U.S. (2009). A comparative analysis of ISSR and RAPD markers for study of genetic diversity in shisham (Dalbergia sissoo). *Plant Molecular Biology Reporter*, 27: 488–495.

Parfitt D.E., Badenes M.L. (1997). Phylogeny of the genus Pistacia as determined from analysis of the chloroplast genome. *Proceedings of the National Academy of Sciences*, 94: 7987–7992.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S., Rafalski A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite)

markers for germplasm analysis. *Molecular Breeding*, 2: 225-238.

Rovira M., Batlle I., Romero M., Vargas F.J. (1995). Isoenzymic identification of Pistacia species. *Acta Horticulturae*, 419: 265-271.

Souframanien J., Gopalakrishna T.A. (2004). Comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics*, 109: 1687-1693.

Whitehouse W.E. (1957). The pistachio nut. A new crop for the Western United States. *Economical Botany*, 11: 281-321.

Williams J.G.K., Kubelik A.R., Levak K.J., Rafalski J.A., Tingey S.V. (1990). DNA polymorphism amplification by arbitrary primers is useful as genetics markers. *Nucleic Acids Research*, 18: 6531-6535.

Ziekiewicz E., Rafalski A., Labuda D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored PCR amplification. *Genomics*, 20: 176-183.

Zohary D. (1996). *The genus Pistacia* L. In: Padulosi S., Caruso T., Barone E. (eds) Taxonomy, distribution, conservation and uses of Pistacia genetic resources. IPGRI, Palermo, Italy, pp 1–11.